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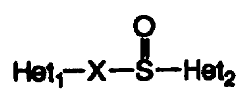
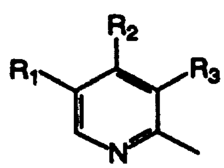
(54) Title: ENANTIOSELECTIVE PREPARATION OF PHARMACEUTICALLY ACTIVE SULFOXIDES BY BIOOXIDATION

(57) Abstract

A compound of formula (II) is prepared either as a single enantiomer or in an enantiomerically enriched form, wherein Het_1 is (a) or (b) and Het_2 is (c) or (d) and X is (e) or (f) wherein N in the benzimidazole moiety means that one of the carbon atoms substituted by R_6-R_9 optionally may be exchanged for an unsubstituted nitrogen atom; R_1, R_2 and R_3 are the same or different and selected from hydrogen, alkyl, alkoxy optionally substituted by fluorine, alkylthio, alkoxyalkoxy, dialkylamino, piperidino, morpholino, halogen, phenylalkyl, phenylalkoxy; R_4 and $R_{4'}$ are the same or different and selected from hydrogen, alkyl, aralkyl; R_5 is hydrogen, halogen, trifluoromethyl, alkyl, alkoxy; R_6-R_9 are the same or different and selected from hydrogen, alkyl, alkoxy, halogen, haloalkoxy, alkylcarbonyl, alkoxycarbonyl, oxazolyl, trifluoroalkyl or adjacent groups R_6-R_9 may complete together with the carbon atoms to which they are attached optionally substitued ring structures; R_{10} is hydrogen or alkoxycarbonyloxymethyl; R_{11} is hydrogen or forms an alkylene chain together with R_3; R_{12} and R_{13} are the same or different and selected from hydrogen, halogen or alkyl, by a method comprising stereoselective biooxidation of the pro-chiral sulfide counterpart compound.



(II)



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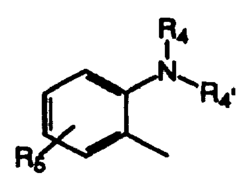

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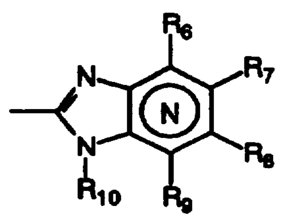

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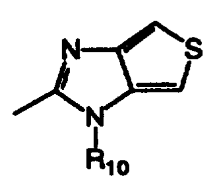

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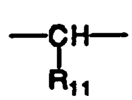

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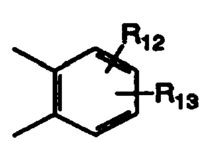

(f)

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Enantioselective preparation of pharmaceutically active sulfoxides by biooxidation

The present invention relates to a method of preparing compounds as defined below, either as a single enantiomer or in an enantiomerically enriched form, by biooxidation of their sulphide equivalents.

Background to the Invention

The racemic form of the compounds prepared by the method of the present invention are known compounds. Some of the compounds are also known in single enantiomeric form. The compounds are active H^+K^+ATPase inhibitors and they, including their pharmaceutically acceptable salts, are effective acid secretion inhibitors, and known for use as antiulcer agents. The compounds, which include the known compounds omeprazole (compound of formula (IIa) below), lansoprazole (compound of formula (IIc) below) and pantoprazole (compound of formula (IIb) below), are known for example from European Patent Specifications EP 5129 and 124495, EP 174726 and EP 166287.

These compounds, being sulfoxides, have an asymmetric centre in the sulfur atom, i.e. exist as two optical isomers (enantiomers). It is desirable to obtain compounds with improved pharmacokinetic and metabolic properties which will give an improved therapeutic profile such as a lower degree of interindividual variation.

The separation of enantiomers of omeprazole in analytical scale is described in e.g. J. Chromatography, 532 (1990), 305-19.Also the separation of enantiomers of compounds, including omeprazole and pantoprazole, is described in German Patent Specification DE 4035455.

Recently there has been a great deal of literature published relating to the synthesis of optically active compounds using biocatalysts. The majority of this work has been aimed at finding routes to single enantiomer forms of pharmaceuticals. The reactions receiving most attention have been those involved in the preparation of esters, acids and alcohols due to the general utility of these functionalities in synthesis and also because the biocatalysts are readily available.

Studies on the synthesis of optically active sulfoxides are relatively rare partly due to the small number of pharmaceuticals containing sulfoxide groups and partly due to the fact that enzymes that react with the sulphur centre are not available commercially. The synthesis of optically active sulfoxides has been described in Holland, H.L. (1988) Chem. Rev. <u>88</u>, 473-483 and Phillips, R.S. and Sheldon W.M., Enzyme Microb. Technol., 1981, Vol. 3, January, 9-18.

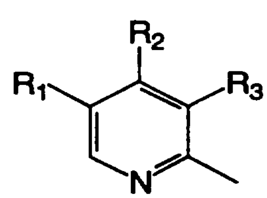
<u>Description of the Invention</u>

According to the present invention there is provided a method of preparing a compound of formula (II) either as a single enantiomer or in an enantiomerically enriched form:

$$\mathrm{Het_1{-}X{-}\overset{\overset{\displaystyle O}{\|}}{S}{-}Het_2} \qquad \text{(II)}$$

wherein

Het $_1$ is



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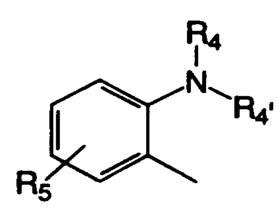

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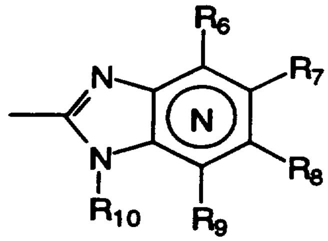
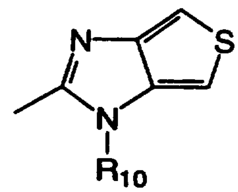
Het 2 is  or 

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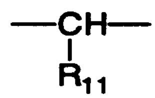
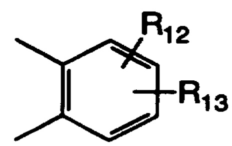
X is  or 

wherein:

N in the benzimidazole moiety means that one of the carbon atoms substituted by R_6-R_9 optionally may be exchanged for an unsubstituted nitrogen atom;

R_1, R_2 and R_3 are the same or different and selected from hydrogen, alkyl, alkoxy optionally substituted by fluorine, alkylthio, alkoxyalkoxy, dialkylamino, piperidino, morpholino, halogen, phenylalkyl, phenylalkoxy;

R_4 and $R_{4'}$ are the same or different and selected from hydrogen, alkyl, aralkyl;

R_5 is hydrogen, halogen, trifluoromethyl, alkyl, alkoxy;

R_6 - R_9 are the same or different and selected from hydrogen, alkyl, alkoxy, halogen, haloalkoxy, alkylcarbonyl, alkoxycarbonyl, oxazolyl, trifluoroalkyl or adjacent groups R_6 - R_9 may complete together with the carbon atoms to which they are attached optionally substituted ring structures;

R_{10} is hydrogen or alkoxycarbonyloxymethyl;

R_{11} is hydrogen or forms an alkylene chain together with R_3;

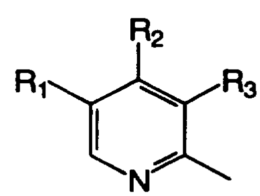
R_{12} and R_{13} are the same or different and selected from hydrogen, halogen or alkyl, which method comprises stereoselective biooxidation of the pro-chiral sulfide counterpart compound.

The compounds of formula (II) are active H^+K^+ATPase inhibitors. By the method of the invention these compounds, which are sulfoxides, are obtained in single enantiomer form or such that one enantiomeric form is present in excess leading to an optically active product, by stereoselective biooxidation of the pro-chiral starting sulfide counterpart compound.

In the above definitions alkyl groups or moieties may be branched or straight chained or comprise cyclic alkyl groups, for example cycloalkylalkyl.

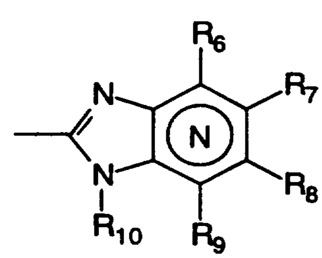
Preferably:

Het $_1$ is



and

Het $_2$ is



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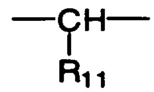
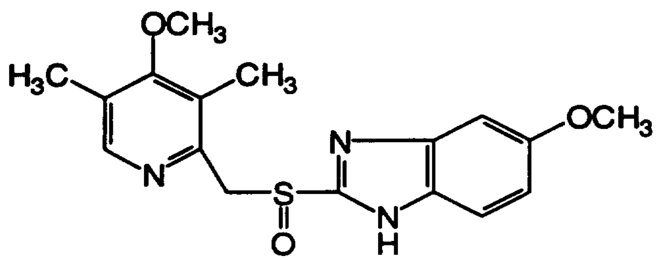
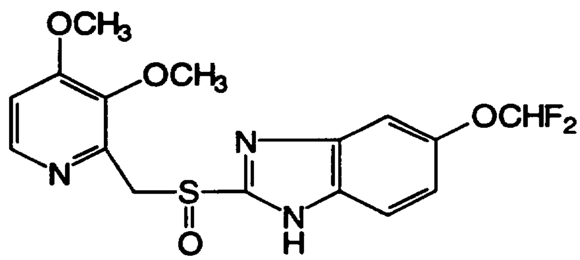
X is 

(wherein R_1, R_2, R_3, R_6 to R_9, R_{10} and R_{11} are as defined above).

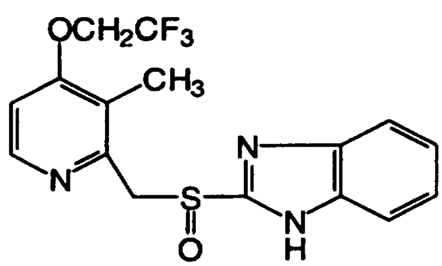
Most preferably the compounds of formula (II) are compounds of the formula (IIa) to (IIe):



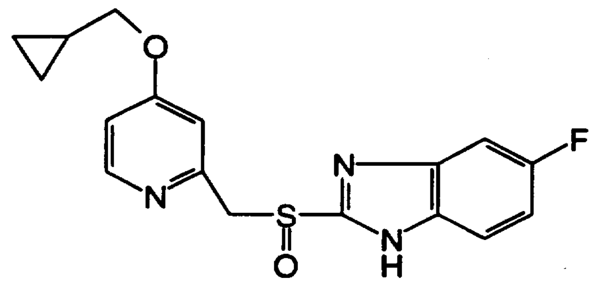
(IIa)



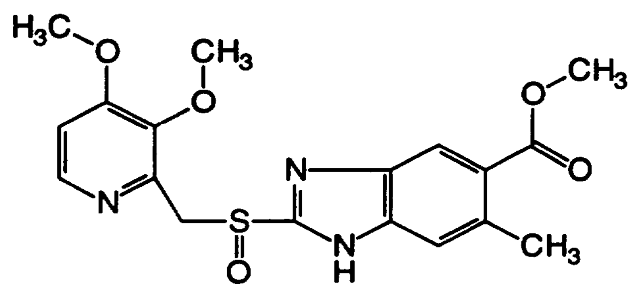
(IIb)



(IIc)

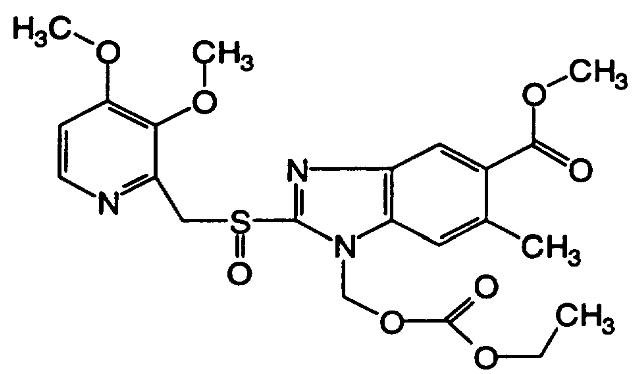


(IId)



(IIe)

An example of a compound of formula (II) wherein R_{10} is alkoxycarbonyloxymethyl is



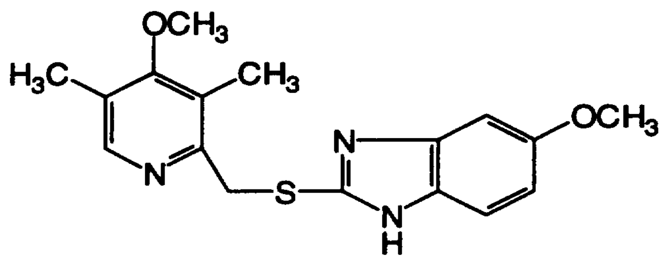
(IIf)

The starting pro-chiral sulfides used in the method of the present invention are of the formula:

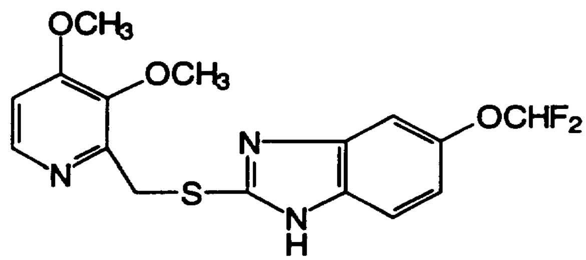
$$Het_1—X—S—Het_2 \qquad (I)$$

wherein Het_1, X and Het_2 are as defined above.

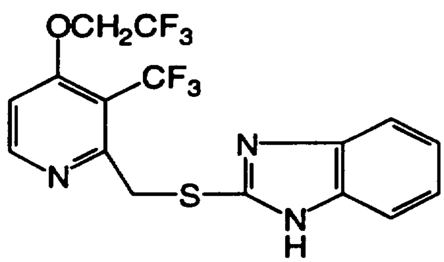
In order to obtain each of the above compounds (IIa)-(IIf), the following starting compounds of formula (Ia) to (If), respectively will be required:



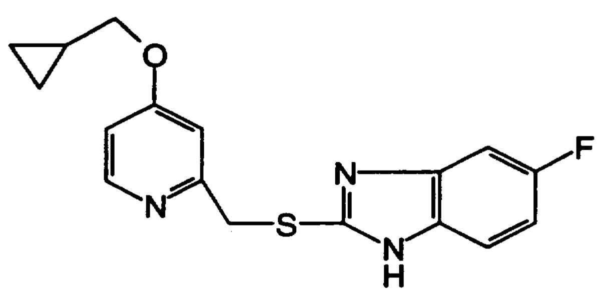
(Ia)



(Ib)



(Ic)



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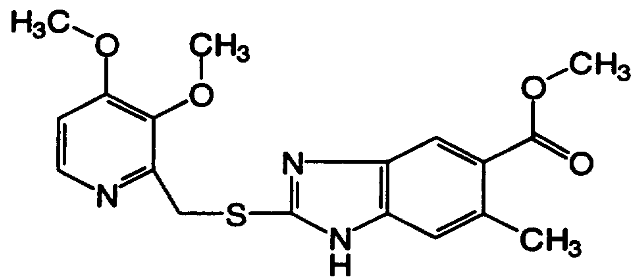
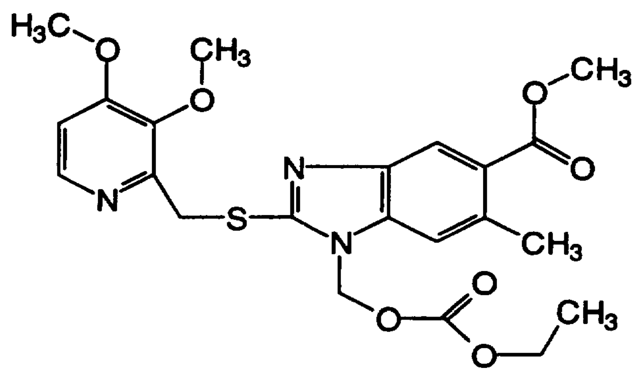


(Ie)



(If)

The compounds prepared by the method of the invention possess a stereogenic (asymmetric) centre which is the sulfur atom which forms the sulfoxide group between the Het_1-X-moiety and the Het_2-moiety.

The stereoselective biooxidation according to the present invention may be carried out using a microorganism or an enzyme system derivable therefrom. Suitable microorganisms may be selected from alkane oxidisers including Arthrobacter petroleophagus, Brevibacterium paraffinolyticum, and Acinetobacter species, alkene oxidisers such as Mycobacterium species, and a variety of fungal species particularly Penicillium species (Penicillium frequentans).

According to one embodiment of the invention the method comprises contacting the pro-chiral sulfide counterpart compound with a microorganism which is

Penicillium frequentans
Rhizopus stolonifer
Cunninghamella elegans
Ustilago maydis
Arthrobacter petroleophagus
Brevibacterium paraffinolyticum
Acinetobacter sp.
Mycobacterium sp.
or Aspergillus niger

Preferably the microorganism is:
Penicillium frequentans BPFC 386, 585, 623, 733
Rhizopus stolonifer BPFC 1581
Ustilago maydis BPFC 1198, 6333
Arthrobacter petroleophagus ATCC 21494
Brevibacterium paraffinolyticum ATCC 21195
Actinetobacter sp. NCIMB 9871
Mycobacterium sp. BPCC 1174, 1178, 1179, 1186, 1187
or Aspergillus niger BPFC 32

The microorganisms may be grown on suitable medium containing an appropriate carbon source such as octane, ethene, cyclohexanone or glucose for example.

The compounds of formula (II) are generally acid labile and thus the use of acid conditions is to be avoided. Generally the method according to the invention may be carried out at a pH of 7.6 to 8, suitably about 7.6, and at temperature of 25-35°C, suitably about 28°C.

The present invention will now be illustrated with reference to the Examples.

EXAMPLE 1

The following microorganisms were screened for sulfoxidation activity against compounds of formula (Ia):

Penicillium frequentans BPFC 386
Penicillium frequentans BPFC 585
Penicillium frequentans BPFC 623
Penicillium frequentans BPFC 733
Rhizopus stolonifer BPFC 1581
Ustilago maydis BPFC 1198
Ustilago maydis BPFC 6333
Arthrobacter petroleophagus ATCC 21494
Brevibacterium paraffinolyticum ATCC 21195
Acinetobacter sp NCIMB 9871
Mycobacterium sp BPCC 1174
Mycobacterium sp BPCC 1178
Mycobacterium sp BPCC 1179
Mycobacterium sp BPCC 1186
Mycobacterium sp BPCC 1187

Growth Conditions

The growth conditions for the above microorganisms were as follows. The following fungi:

Penicillium frequentans BPFC 386
Penicillium frequentans BPFC 585
Penicillium frequentans BPFC 623
Penicillium frequentans BPFC 733

Rhizopus stolonifer BPFC 1581
Ustilago maydis BPFC 1198
Ustilago maydis BPFC 6333

were grown in 200 ml of sterile liquid medium (I) with the composition of (per litre) K_2HPO_4 (1.9g), NaH_2PO_4 $2H_2O$ (2.02g), ammonium sulfate (1.8g), magnesium sulfate (0.2g), ferric chloride (0.97 mg), and trace elements solution (1 ml) pH 7.2. The composition of the trace elements solution used was as follows (in g/l):

$CuSO_4 . 5H_20$	0.02
$MnS0_4 . 4H_20$	0.1
$ZnS0_4 . 7H_20$	0.1
$CaC0_3$	1.8

The above medium was supplemented with 0.2% w/v yeast extract and 2.2% w/v glucose. The medium contained in 1L baffled flasks was inoculated either by adding a suspension of spores in sterile distilled water or by the addition of a plug of agar containing the fungi from a Sabouraud Dextrose plate. Fungi were grown at 28°C on a rotary shaker at 150 rpm for 48 hours. With the exception of Ustilago maydis, the fungal biomass obtained from liquid culture was harvested by filtration on a Whatman Grade 113 filter paper and washed on the filter with 50 mM sodium phosphate buffer, pH7.6. Ustilago maydis was harvested by centrifuging for 20 minutes at 8,000 rpm and 4°C. The biomass was washed by resuspending in 50 mM sodium phosphate buffer, pH 7.6 and centrifuging as above.

The bacteria were grown with the sources of carbon shown in Table 1:

TABLE 1

Microorganism	Carbon Source
Arthrobacter petroleophagus ATCC 21494	Octane
Brevibacterium paraffinolyticum ATCC 21195	Octane
Acinetobacter sp NCIMB 9871	Cyclohexanone
Mycobacterium sp BPCC 1174, 1178, 1179, 1186, 1187	Ethene

The growth of Acinetobacter sp. NCIMB 9871 on cyclohexanone was performed in 100 ml of liquid medium (I) in a 500 ml baffled flask containing a centre well. Cyclohexanone was placed in the centre well. The microorganism was grown at 28°C on a rotary shaker at 150 rpm for 24-48 hours.

Growth of Arthrobacter petroleophagus ATCC 21494 and Brevibacterium paraffinolyticum ATCC 21195 on octane was performed in 200 ml of liquid medium (I) containing 0.2% w/v yeast extract in a 1 L baffled flask. Octane (1ml) was added directly to the medium without sterilization. The above microorganisms were grown at 28°C on a rotary shaker at 150 rpm for 24-48 hours.

Mycobacterium sp BPCC 1174, 1178, 1179, 1186 and 1187 were grown in 500 ml liquid medium (I) in a 2L non-baffled flask fitted with a rubber bung. The flask was partially evacuated and then charged with ethene. Growth was conducted at 28°C on a rotary shaker at 150 rpm for 7 days.

Growth of Arthrobacter petroleophagus ATCC 21494 and Brevibacterium paraffinolyticum ATCC 21195 was also performed on glucose. Each microorganism was inoculated into 200 ml medium (I) containing 0.2% w/v yeast extract and 2.2% w/v glucose. Growth was performed at 28°C on a rotary shaker at 150 rpm for 24-48 hours.

All bacteria were harvested from liquid medium by centrifuging at 8,000 rpm and 4°C for 20 minutes. Cells were washed by resuspending in 50 mM sodium phosphate buffer, pH 7.6 followed by centrifuging as above.

Biooxidation Reactions

Biotransformations were performed for each microorganism in 50mM sodium phosphate buffer, pH 7.6 with 5-10 g/l dry cell weight and a substrate concentration of 1 g/l. The cells were incubated with the compound of formula (Ia) on a rotary shaker at 28°C for 18-20 hours.

Samples were removed from the biotransformation and either centrifuged or filtered to remove biomass and analysed directly.

Detection of Products

The biooxidation of the compound of formula (Ia) was followed by reverse phase HPLC on a Spherisorb S5-ODS2 reverse phase column eluted with a 50:50 mixture of acetonitrile and 25mM sodium phosphate buffer, pH 7.6 at a flow rate of 0.8 ml/min. Under such conditions the compounds of formulae (IIa) and (Ia) were well resolved with retention times of 5.2 and 9.8 minutes respectively. Both compounds were detected at a wavelength of 300 nm.

The enantiomeric composition of the compound of formula (IIa) formed was investigated by the following method. After removal of biomass the aqueous media was extracted with two volumes of ammonia saturated dichloromethane. The pooled organic extracts were dried over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure to afford a pale brown solid. Then the enantiomeric composition of sulfoxide was determined by chiral HPLC on a Chiralpak AD Column under the following conditions:

Column Chiralpack AD 250 mm x 4.6 mm interior

diameter with 50 mm guard column

Eluent Hexane:Ethanol:Methanol (40:55:5% V/V)
Flow 1.0 ml/min
Injection Volume 20µl
Wavelength 300 nm
Retention times
Compound of formula (Ia) 5.1 min
Compound of formula (IIa):
(+) Enantiomer 8.5 min
(-) Enantiomer 13.4 min

The following results were obtained:

TABLE 2

Microorganism	Compound of Formula (IIa) (ppm)	Enantiomeric excess (%)	Enantiomer ((+) or (-))
Penicillium frequentans BPFC 386	23	>99	(-)
Penicillium frequentans BPFC 585	2.1	>99	(-)
Penicillium frequentans BPFC 623	3.0	95	(-)
Penicillium frequentans BPFC 733	2.6	87	(-)
Rhizopus stolonifer BPFC 1581	3.0	56	(-)
Ustilago maydis BPFC 1198	8.0	88	(-)
Ustilago maydis BPFC 6333	34.0	99	(-)
Arthrobacter petroleophagus ATCC 21494	24.0	96	(-)
Brevibacterium paraffinolyticum ATCC 21195	13.0	>99	(-)
Acinetobacter sp NCIMB 9871	0.4	17	(-)
Mycobacterium sp BPCC 1174	10.0	97	(-)
Mycobacterium sp BPCC 1178	3.3	93	(-)
Mycobacterium sp BPCC 1179	9.0	96	(-)
Mycobacterium sp BPCC 1186	11.0	97	(-)
Mycobacterium sp BPCC 1187	6.0	96	(-)

The enantiomeric excess value gives an indication of the relative amounts of each enantiomer obtained. The value is the difference between the relative percentages for the two enantiomers. Thus, for example, when the percentage of the (-) enantiomer of the formed sulfoxide is 97.5% and the percentage for the (+) enantiomer is 2.5%, the enantiomeric excess for the (-) enantiomer is 95%.

With Arthrobacter petroleophagus ATCC 21494 and Brevibacterium paraffinolyticum ATCC 21195 the stereoselectivity of the biooxidation was unaffected by the choice of carbon source used for growth (octane and glucose).

EXAMPLE 2

Compounds of formula (Id) and (Ie) were screened against a range of microorganisms for the production of the corresponding sulfoxides. The growth of microorganisms and subsequent biotransformations were performed as in Example 1 except that the reaction times were as listed in Tables 5 and 6. Aspergillus niger BPFC 32 was grown in the same way as the fungi were grown in Example 1.

Detection of Products

The biooxidation of the compounds of formula (Id) and (Ie) was followed by reverse phase HPLC as in Example 1 except that the retention times were as follows:

TABLE 3

Compound of formula	Retention time (min)
Id	13.7
IId	5.0
Ie	9.4
IIe	4.3

The enantiomeric composition of the compounds of formula (IId) and (IIe) was investigated by the method of Example 1 except in the chiral HPLC the solvent compositions, flow rates and retention times were as follows:

TABLE 4

Compound of formula	Solvent Composition	Flow rate (ml/min)	Retention Time
IId	Hexane/Ethanol (70:30% v/v)	1.0	12.9 (Enantiomer A) 21.7 (Enantiomer B)
	Hexane/Ethanol/Methanol (40:55:5% v/v)	1.0	7.4 (Enantiomer A) 10.6 (Enantiomer B)
IIe	Hexane/Ethanol (70:30% v/v)	1.0	26.0 (Enantiomer A) 30.5 (Enantiomer B)

In Table 4 the first enantiomer eluted is referred to as enantiomer A and second as enantiomer B. The results are summarised in Tables 5 and 6.

TABLE 5

Microorganism	Reaction time (h)	Aqueous concentration (PPM)		E.e. %	Enantiomer
		Compound of formula (Id)	Compound of formula (IId)		
Mycobacterium sp. BPCC 1174	42	5	16.7	>99	A
Mycobacterium sp. BPCC 1178	42	5.9	14.4	>99	A
Mycobacterium sp. BPCC 1179	42	6.6	17.4	>99	A
*Mycobacterium sp.*BPCC 1186	42	4.8	42	>99	A
*Mycobacterium sp.*BPCC 1187	42	7.4	18.3	>99	A
Arthrobacter petroleophagus ATCC 21494	42	3.5	6.6	>99	A
Brevibacterium paraffinolyticum ATCC 21195	42	2.6	21.7	>99	A
Ustilago maydis BPFC 1198	18	6.7	45	>99	A
Ustilago maydis BPFC 6333	18	4.6	43	>99	A
Aspergillus niger BPFC 32	42	5.6	2.7	-	-
Penicillium frequentans BPFC 386	18	5	0	-	-
Penicillium frequentans BPFC 585	48	5.2	0	-	-
Penicillium frequentans BPFC 623	48	4.5	0	-	-
Penicillium frequentans BPFC 733	18	3.5	0	-	-

(E.e. means Enantiomeric excess)

TABLE 6

Microorganism	Reactiontime (h)	Aqueous concentration (PPM)		E.e (%)	Enantiomer
		Compound of formula (Ie)	Compound of formula (IIe)		
Mycobacterium sp. BPCC 1179	42	1.6	3.3	>99	A
Arthrobacter petroleophagus ATCC 21494	42	3.2	0	-	-
Brevibacterium paraffinolyticum ATCC 21195	72	4.0	1.6	-	-
Ustilago maydis BPFC 1198	18	2.3	0	-	-
Ustilago maydis BPFC 6333	72	3.2	0	-	-
Asergillus niger BPFC 32	72	3.7	9.2	-	-
Penicillium frequentans BPFC 386	72	3.1	0.5	-	-
Penicillium frequentans BPFC 585	48	3.2	3.2	-	-
Penicillium frequentans BPFC 623	48	2.9	1.5	83.4-	B
Penicillium frequentans BPFC 733	18	3.2	0		-

The oxidation of the compound of formula (Id) produced in all cases the "A" enantiomer of the compound of formula (IId) in excellent enantiomeric excess but in low yield. The four strains of Penicillium frequentans, previously shown to oxidise the compound of formula (Ia), failed to oxidise the compound of formula (Id).

The oxidation of the compound of formula (Ie) produced fewer results. This compound proved to be particularly insoluble making the detection of product difficult. Whilst in a number of cases sulfoxide was produced, its concentration was too low to determine the enantiomeric excess. However two results were obtained with Mycobacterium sp. and Penicillium frequentans both affording sulfoxide of high enantiomeric excess but interestingly of opposite stereoselectivity.

EXAMPLE 3

The microorganisms listed in Table 9 below were screened for sulfoxidation activity against compounds of formula (Ib). They were grown under the same condition as in Examples 1 and 2.

Biotransformations were performed following the protocol of Example 1 except that the dry cell weight was increased to approximately $20gL^{-1}$ and the reaction time was extended.

Detection of Products

The biooxidation of the compound of formula (Ib) was followed by reverse phase HPLC as in Example 1 except that the retention times were as follows:

TABLE 7

Compound of formula	Retention time (min)
Ib IIb	8.1 4.2

The enantiomeric composition of the compound of formula (IIb) was investigated by the method of Example 1 except in the chiral HPLC the solvent composition, flow rate and retention time were as follows:

TABLE 8

Solvent composition	Flow Rate (ml/min)	Retention times (min)
Hexane/ethanol (70:30%)	1.0	32.3 (Enantiomer A) 36.6 (Enantiomer B)

In Table 8 the first enantiomer eluted is referred to as enantiomer A and the second as enantiomer B.

The results are summarised in the following table:

TABLE 9

Microorganism	Reaction time (h)	Aqueous concentration (PPM)		E.e (%)	Enantio-mer
		Compound of formula (Ib)	Compound of formula (IIb)		
Mycobacterium sp. BPCC 1178	72	8.6	3.4	8.2	B
Brevibacterium paraffinolyticum ATCC 21195	72	8.4	4.0	26.6	B
Ustilago maydis BPFC 6333	72	8.2	4.3	>99	A
Aspergillus niger BPFC 32	72	5.6	28.0	>99	A
Penicillium frequentans BPFC 386	72	8.4	4.5	-	-
Penicillium frequentans BPFC 585	48	6.5	11.4	-	-
Penicillium frequentans BPFC 623	48	7.7	6.5	-	-

(E.e. means enantiomeric excess)

The microorganisms listed in Table 9 were also screened under identical conditions for sulfoxidation of the compound of formula (Ic) but no product of formula (IIc) could be detected.

Deposits Of Microorganisms

The following microorganisms were deposited at the National Collections of Industrial and Marine Bacteria Ltd (NCIMB), 23 St. Machar Drive, Aberdeen, Scotland AB2 1RY on 25 November 1994:

1. Mycobacterium sp BPCC 1174
   Accession No. NCIMB 40695
2. Mycobacterium sp BPCC 1178
   Accession No. NCIMB 40696
3. Mycobacterium sp BPCC 1179
   Accession No. NCIMB 40697
4. Mycobacterium sp BPCC 1186
   Accession No. NCIMB 40698
5. Mycobacterium sp BPCC 1187
   Accession No. NCIMB 40699

   The following microorganisms were deposited at the International Mycological Institute (IMI), Bakeham Lane, Englefield Green, Egham, Surrey TW20 9TY, England on 28 November 1994:

6. Penicillium frequentans BPFC 386
   Accession No. IMICC 364802
7. Penicillium frequentans BPFC 585
   Accession No. IMICC 364801
8. Penicillium frequentans BPFC 623

Accession No. IMICC 364800

9. Penicillium frequentans BPFC 733
Accession No. IMICC 364799

10. Rhizopus stolonifer BPFC 1581
Accession No. IMICC 364798

11. Ustilago maydis BPFC 1198
Accession No. IMICC 364797

12. Ustilago maydis BPFC 6333
Accession No. IMICC 364796

13. Asperigillus niger BPFC 32
Accession No. IMICC 364795

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 20, line 15.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution

The National Collections of Industrial and Marine Bacteria Limited

Address of depositary institution *(including postal code and country)*

23 St Machar Drive
ABERDEEN AB2 1RY
Scotland, United Kingdom

Date of deposit	Accession Number
November 25, 1994	NCIMB 40695

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism(s) be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. EPC Rule 28(4), U.K. Rule 17(3), Australian Regulation 3.25(3) and generally similar provisions mutatis mutandis for any other designated state.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 20, line 17.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet [X]

Name of depositary institution

The National Collections of Industrial and Marine Bacteria Limited

Address of depositary institution *(including postal code and country)*

23 St Machar Drive
ABERDEEN AB2 1RY
Scotland, United Kingdom

Date of deposit	Accession Number
November 25, 1994	NCIMB 40696

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet [ ]

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism(s) be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. EPC Rule 28(4), U.K. Rule 17(3), Australian Regulation 3.25(3) and generally similar provisions mutatis mutandis for any other designated state.

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 20, line 19.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution

The National Collections of Industrial and Marine Bacteria Limited

Address of depositary institution *(including postal code and country)*

23 St Machar Drive
ABERDEEN AB2 1RY
Scotland, United Kingdom

Date of deposit	Accession Number
November 25, 1994	NCIMB 40697

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism(s) be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. EPC Rule 28(4), U.K. Rule 17(3), Australian Regulation 3.25(3) and generally similar provisions mutatis mutandis for any other designated state.

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 20 **, line** 21 .

B. IDENTIFICATION OF DEPOSIT — **Further deposits are identified on an additional sheet** [X]

Name of depositary institution

The National Collections of Industrial and Marine Bacteria Limited

Address of depositary institution ***(including postal code and country)***

23 St Machar Drive
ABERDEEN AB2 1RY
Scotland, United Kingdom

Date of deposit	Accession Number
November 25, 1994	NCIMB 40698

C. ADDITIONAL INDICATIONS ***(leave blank if not applicable)*** — **This information is continued on an additional sheet** [ ]

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism(s) be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. EPC Rule 28(4), U.K. Rule 17(3), Australian Regulation 3.25(3) and generally similar provisions mutatis mutandis for any other designated state.

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 20, line 23.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution

The National Collections of Industrial and Marine Becteria Limited

Address of depositary institution *(including postal code and country)*

23 St Machar Drive
ABERDEEN AB2 1RY
Scotland, United Kingdom

Date of deposit	Accession Number
November 25, 1994	NCIMB 40699

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism(s) be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. EPC Rule 28(4), U.K. Rule 17(3), Australian Regulation 3.25(3) and generally similar provisions mutatis mutandis for any other designated state.

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 20, line 28.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet [X]

Name of depositary institution

International Mycological Institute

Address of depositary institution *(including postal code and country)*

Bakeham Lane
Egham
Surrey
TW20 9TY, England, UK

Date of deposit	Accession Number
November 28, 1994	IMICC 364802

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet [ ]

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism(s) be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. EPC Rule 28(4), U.K. Rule 17(3), Australian Regulation 3.25(3) and generally similar provisions mutatis mutandis for any other designated state.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

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A. The indications made below relate to the microorganism referred to in the description on page 20, line 30.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution

International Mycological Institute

Address of depositary institution *(including postal code and country)*

Bakeham Lane
Egham
Surrey
TW20 9TY, England, UK

Date of deposit	Accession Number
November 28, 1994	IMICC 364801

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism(s) be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. EPC Rule 28(4), U.K. Rule 17(3), Australian Regulation 3.25(3) and generally similar provisions mutatis mutandis for any other designated state.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 21, line 1.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet [X]

Name of depositary institution

International Mycological Institute

Address of depositary institution *(including postal code and country)*

Bakeham Lane
Egham
Surrey
TW20 9TY, England, UK

Date of deposit	Accession Number
November 28, 1994	IMICC 364800

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet [ ]

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism(s) be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. EPC Rule 28(4), U.K. Rule 17(3), Australian Regulation 3.25(3) and generally similar provisions mutatis mutandis for any other designated state.

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 21, line 3.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet [X]

Name of depositary institution

International Mycological Institute

Address of depositary institution *(including postal code and country)*

Bakeham Lane
Egham
Surrey
TW20 9TY, England, UK

Date of deposit	Accession Number
November 28, 1994	IMICC 364799

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet [ ]

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism(s) be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. EPC Rule 28(4), U.K. Rule 17(3), Australian Regulation 3.25(3) and generally similar provisions mutatis mutandis for any other designated state.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 21, line 5.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet [X]

Name of depositary institution

International Mycological Institute

Address of depositary institution *(including postal code and country)*

Bakeham Lane
Egham
Surrey
TW20 9TY, England, UK

Date of deposit	Accession Number
November 28, 1994	IMICC 364798

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet [ ]

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism(s) be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. EPC Rule 28(4), U.K. Rule 17(3), Australian Regulation 3.25(3) and generally similar provisions mutatis mutandis for any other designated state.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 21 **, line** 7 .

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution

International Mycological Institute

Address of depositary institution *(including postal code and country)*

Bakeham Lane
Egham
Surrey
TW20 9TY, England, UK

Date of deposit	Accession Number
November 28, 1994	IMICC 364797

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism(s) be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. EPC Rule 28(4), U.K. Rule 17(3), Australian Regulation 3.25(3) and generally similar provisions mutatis mutandis for any other designated state.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 21, line 9.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet [X]

Name of depositary institution
International Mycological Institute

Address of depositary institution *(including postal code and country)*
Bakeham Lane
Egham
Surrey
TW20 9TY, England, UK

Date of deposit	Accession Number
November 28, 1994	IMICC 364796

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet [ ]

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism(s) be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. EPC Rule 28(4), U.K. Rule 17(3), Australian Regulation 3.25(3) and generally similar provisions mutatis mutandis for any other designated state.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 21, line 11.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet [X]

Name of depositary institution

International Mycological Institute

Address of depositary institution *(including postal code and country)*

Bakeham Lane
Egham
Surrey
TW20 9TY, England, UK

Date of deposit	Accession Number
November 28, 1994	IMICC 364795

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet [ ]

In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism(s) be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. EPC Rule 28(4), U.K. Rule 17(3), Australian Regulation 3.25(3) and generally similar provisions mutatis mutandis for any other designated state.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

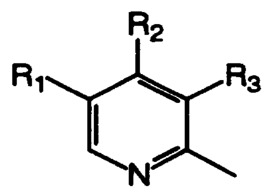
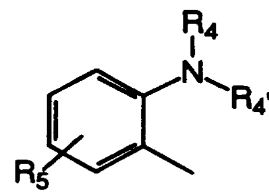
For receiving Office use only	For International Bureau use only
[X] This sheet was received with the international application	[ ] This sheet was received by the International Bureau on:
Authorized officer Marie Hell	Authorized officer

CLAIMS

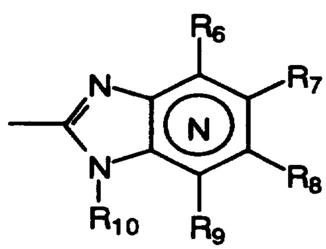
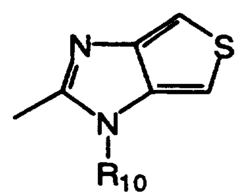
1. A method of preparing a compound of formula (II) either as a single enantiomer or in an enantiomerically enriched form:

$$Het_1-X-\overset{\overset{\Large O}{\|}}{S}-Het_2 \qquad (II)$$

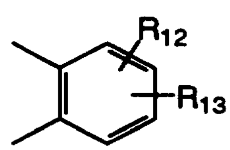
wherein:

Het 1 is  or 

and

Het 2 is  or 

and

X is $-\underset{\displaystyle R_{11}}{\underset{|}{C}}H-$ or 

wherein:

N in the benzimidazole moiety means that one of the carbon atoms substituted by R_6-R_9 optionally may be exchanged for an unsubstituted nitrogen atom;

R_1, R_2 and R_3 are the same or different and selected from hydrogen, alkyl, alkoxy optionally substituted by fluorine, alkylthio, alkoxyalkoxy, dialkylamino, piperidino, morpholino, halogen, phenylalkyl, phenylalkoxy;

R_4 and $R_{4'}$ are the same or different and selected from hydrogen, alkyl, aralkyl;

R_5 is hydrogen, halogen, trifluoromethyl, alkyl, alkoxy;

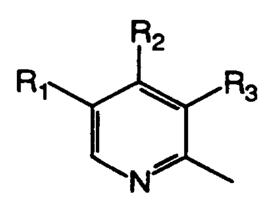
R_6 - R_9 are the same or different and selected from hydrogen, alkyl, alkoxy, halogen, haloalkoxy, alkylcarbonyl, alkoxycarbonyl, oxazolyl, trifluoroalkyl or adjacent groups R_6 - R_9 may complete together with the carbon atoms to which they are attached optionally substituted ring structures;

R_{10} is hydrogen or alkoxycarbonyloxymethyl;

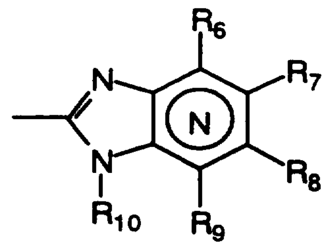
R_{11} is hydrogen or forms an alkylene chain together with R_3;

R_{12} and R_{13} are the same or different and selected from hydrogen, halogen or alkyl; which method comprises stereoselective biooxidation of the pro-chiral sulfide counterpart compound.

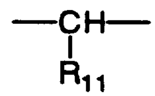
2. A method according to claim 1 wherein:

Het $_1$ is 

and

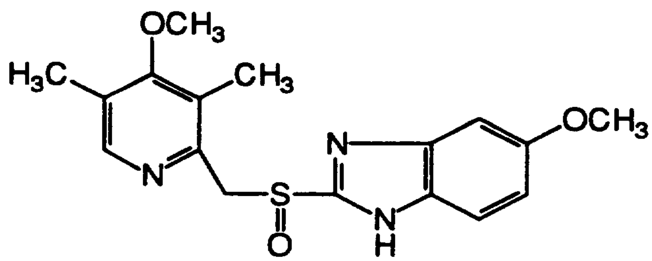
Het $_2$ is 

and

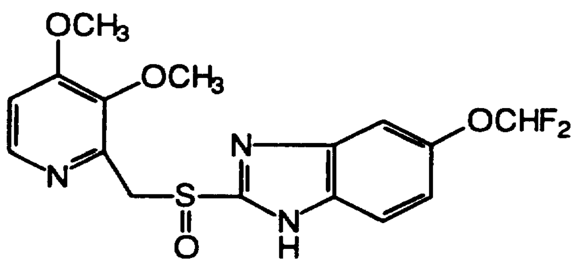
X is 

wherein R_1, R_2, R_3, R_6-R_9, R_{10} and R_{11} are as defined in claim 1.

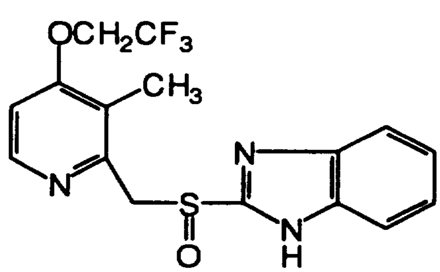
3. A method to claim 1 or 2 wherein the compound of formula (II) is a compound of formula:



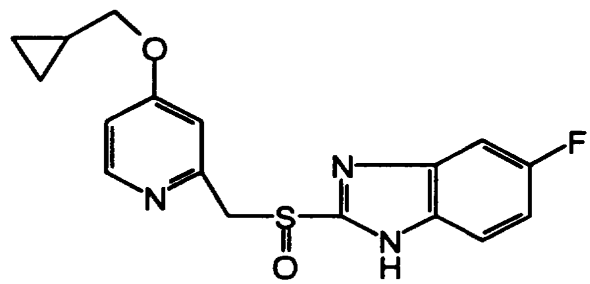
(IIa)



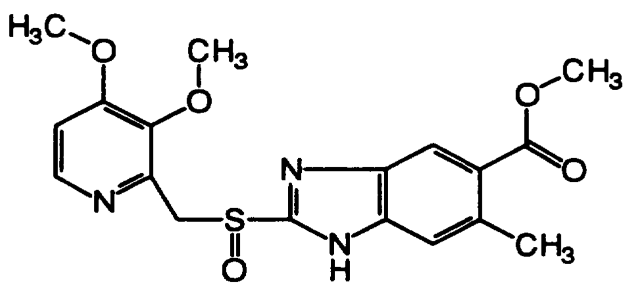
(IIb)



(IIc)



(IId)



(IIe)

4. A method according to any one of the previous claims wherein a single enantiomer of the compound of formula (II) is prepared.

5. A method according to claim 3 wherein there is prepared a compound of formula (IIa) and the biooxidation is carried out with a microorganism which is

Penicillium frequentans
Brevibacterium paraffinolyticum or
Mycobacterium sp.

6. A method according to claim 3 wherein there is prepared a compound of formula (IIb) and the biooxidation is carried out with a microorganism which is:

Aspergillus niger or
Ustilago maydis.

7. A method according to claim 3 wherein there is prepared a compound of formula (IId) and the biooxidation is carried out with a microorganism which is

Mycobacterium sp.
Arthrobacter petroleophagus
Brevibacterium paraffinolyticum or
Ustilago maydis.

8. A method according to claim 3 wherein there is prepared a compound of formula (IIe) and the bioxidation is carried out with a microorganism which is:

Mycobacterium sp.
Penicillium frequentans

9. A method according to claim 1 substantially as described in any one of the Examples.

10. A compound of formula II, as a single enantiomer or in enantiomerically enriched form, as defined in claim 1 prepared by the method claimed in any one of claims 1 to 9.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 95/01415

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12P 11/00, C07D 401/12
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, IFIPAT, CA, MEDLINE, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0005129 A1 (AB HÄSSLE), 31 October 1979 (31.10.79) --	1-4
A	Chem. Rev., Volume 88, 1988, H.L. Holland, "Chiral Sulfoxidation by Biotransformation of Organic Sulfides" page 473 - page 485 --	1-10
A	Drug Metabolism and Disposition, Volume 21, No 4, 1993, J.R. Cashman et al., "Chemical, Enzymatic and Human Enantioselective S-Oxygenation of Cimetidine" page 587 - page 597 --	1-10

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" erlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 March 1996	21 -03- 1996
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Gerd Strandell Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 95/01415

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Enzyme Microb. Technol., Volume 3, 1981, R.S. Philips, S.W. May, "Enzymatic sulphur oxygenation reactions" page 9 - page 18	1-10

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INTERNATIONAL SEARCH REPORT

Information on patent family members

05/02/96

Inte...ational application No.
PCT/SE 95/01415

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A1- 0005129	31/10/79	SE-T3-	0005129	
		AT-B-	374471	25/04/84
		AT-B-	374472	25/04/84
		AT-B-	374473	25/04/84
		AT-B-	375365	25/07/84
		AT-B-	389995	26/02/90
		AU-B,B-	529654	16/06/83
		AU-A-	4602779	18/10/79
		CA-A-	1127158	06/07/82
		CA-A-	1129417	10/08/82
		JP-C-	1312930	28/04/86
		JP-C-	1504537	13/07/89
		JP-A-	54141783	05/11/79
		JP-A-	58192880	10/11/83
		JP-B-	60034956	12/08/85
		JP-B-	63053191	21/10/88
		LU-A-	88307	04/05/94
		SE-A-	7804231	15/10/79
		SU-A,A-	895292	30/12/81
		US-A-	4255431	10/03/81
		US-A-	4337257	29/06/82
		US-A-	4508905	02/04/85